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High-Resolution Neutron Diffraction Data Collected on the Protein Crystallography Station at LANSCE Exceed Researchers' Expectations

The protein D-xylose isomerase is an enzyme that catalyzes the conversion of D-xylose to D-xylulose and glucose to fructose by hydrogen-atom transfer. Used extensively by the sweetener industry for the production of high-fructose corn syrup, this protein plays an important economic role in the multi-billion dollar soft-drink market and is the subject of intense research in many other areas. Studying the enzyme that produces sugar could enhance our understanding of the mechanism behind proton transfer and lead to the discovery of highly specific disease- and cancer-fighting drugs and innovations in nanotechnology. However, scientists must first find a way to locate the hydrogen atoms within D-xylose isomerase, not just the heavier atoms found by x-ray diffraction. Vital information about the structure, function, and, in particular, the mechanism of the hydrogen-atom transfer can be obtained for this enzyme by neutron diffraction studies. The catalytic mechanism of hydrogen-atom transfer is currently believed to involve a metal ion-bound water molecule, a mechanism that is thought to be used by many important proteins involved in human disease. Neutrons are a unique tool for structural determination of biological materials because light atoms (hydrogen atoms) diffract neutrons as strongly as do the other atoms commonly found in macromolecules (carbon, nitrogen, oxygen, and phosphorus). By contrast, in x-ray diffraction, the hydrogen atoms diffract very weakly. Therefore, these mechanistic details can only be determined by neutron-diffraction techniques (Fig. 1).

Gerard Bunick and Leif Hanson from Oak Ridge National Laboratory and Jenny Glusker, Amy Katz, and H. L. Carrell from Fox Chase Cancer Center are participating in a long-running National Institutes of Health structural biology project to determine the hydrogen atom positions of D-xylose isomerase by high-resolution x-ray crystallography and neutron diffraction. Scientists already know the atomic positions of the non-hydrogen atoms in this protein. However, since about 50% of the atoms in this protein are hydrogen atoms, knowing the exact atomic positions of the hydrogen atoms is critical to understanding its structure and function. D-xylose isomerase is a good test case in these current experiments, which were specifically designed to compare the results of x-ray crystallography and neutron diffraction. A major advantage of using neutron diffraction in this study is that neutrons do not cause extensive radiation damage to the crystal. In previous experiments, both the Oak Ridge and Fox Chase teams found that xylose isomerase crystals cannot diffract at room temperature beyond 1.4 Å with x-rays, and this resolution is not sufficient to reveal the positions of hydrogen atoms. Neutrons, however, can be used to locate hydrogen atoms even at moderate resolutions (around 2.2 Å) — thus providing a strong scientific justification for using neutron diffraction in biological studies.

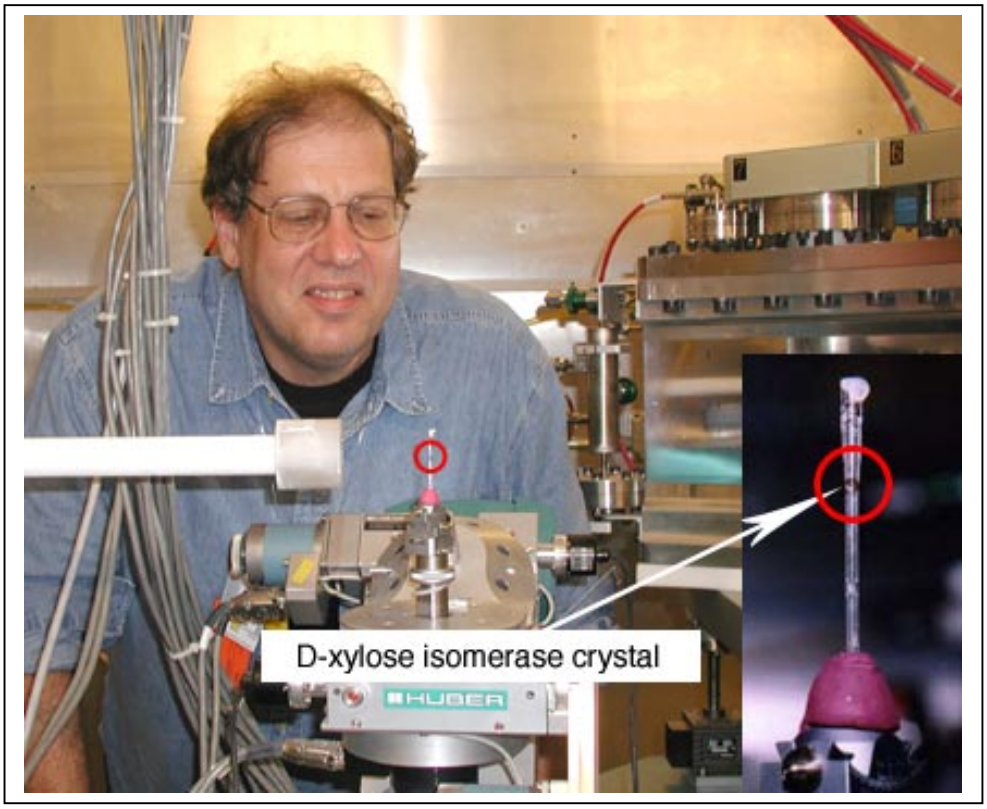

Fig. 1. *Leif Hanson of Oak Ridge National Laboratory, who believes that "proteins are nature's way of ordering water to do its jobs for it," examines the crystal sample of the D-xylose isomerase protein used in recent experiments on the PCS at Lujan Center. On the right, next to Leif, is the PCS detector array. This sample of D-xylose isomerase is the largest biological molecule ever studied at high resolution using time-of-flight neutron diffraction.*

As the first external users on the newly commissioned Protein Crystallography Station (PCS), built and run by the Bioscience Division at Los Alamos National Laboratory, the Oak Ridge/Fox Chase team performed pioneering experiments on D-xylose isomerase. D-xylose isomerase is the largest biological molecule (with unit-cell lengths around 100 Å) ever studied at high resolution using neutron diffraction and one of the first proteins to be studied using time-of-flight techniques (Fig. 2). The study is being done in response to a request by the Department of Energy Office of Biological and Environmental Research to provide clear evidence for neutron diffraction as a tool for macromolecular structure/function research. This work also has far-reaching implications for the development of neutron-diffraction techniques and tools needed for implementing these types of studies at the future Spallation Neutron Source. The primary goal of the experiments on the PCS, however, was to collect neutron-diffraction data on a crystal sample of D-xylose isomerase to compare the structural information obtained by this technique with that derived from x-ray data taken at the Advanced Photon Source (APS) at Argonne National Laboratory.

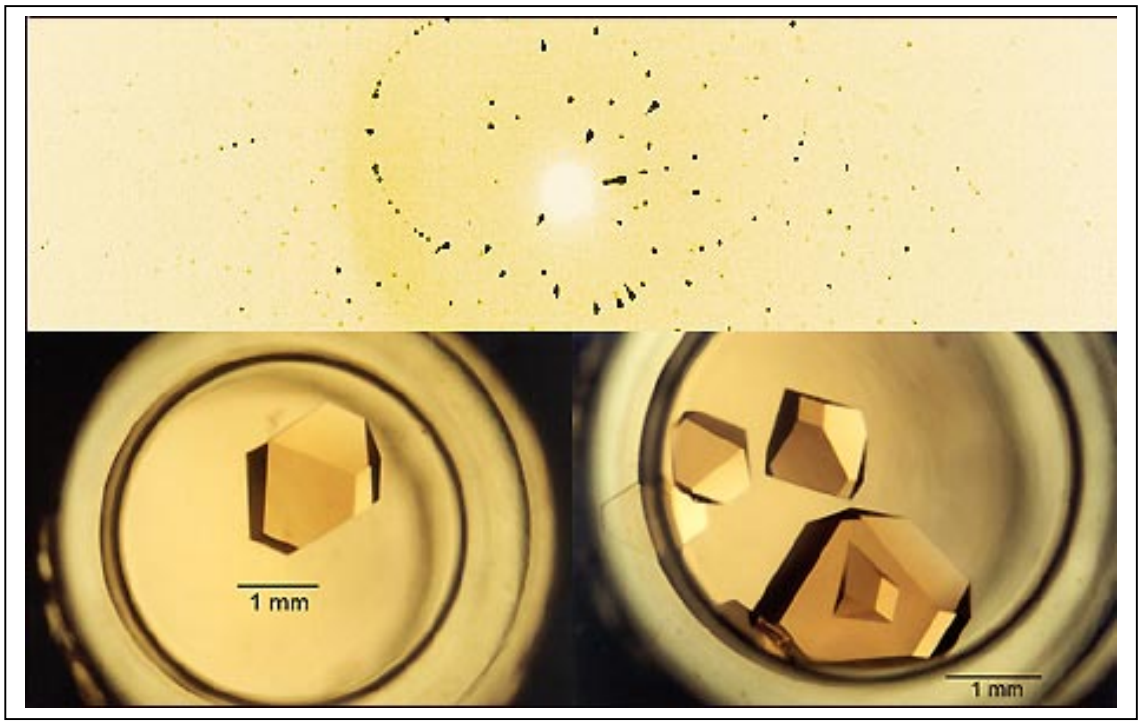

Fig. 2. *The top image is the neutron-diffraction data taken on the PCS of one of the D-xylose isomerase protein crystals shown in the bottom right image. This sample was provided by Genencor International, Inc., a worldwide supplier of enzymes and other biochemicals for industrial use. These crystals were grown at Oak Ridge National Laboratory in a counter diffusion cell, which is a NASA device originally developed for microgravity protein crystallization.*

The work on D-xylose isomerase, a single protein, will lead to similar studies currently beyond technical capabilities to characterize the roles that hydrogen atoms play in larger biological systems, such as the ribosome — the cellular organelle *workbench* for the construction of all proteins. A determination of the complete structure of the ribosome could shed light on the basic process of metabolism and enable, for example, the design of better, more specific, antibiotics. (It will take a really large instrument to see all the atoms in one of nature's important tools.) Other multi-component biological assemblages, such as gene modulation complexes (i.e., DNA plus proteins), may also yield structural information that will ultimately offer insight into the processes of gene expression/silencing and of mutation, carcinogenesis, and low-dose radiation effects.

Scientists at Fox Chase Cancer Center are interested in what they believe is the metal *ion-mediated* ionization of water as the enzymatic mechanism of D-xylose isomerase. The result is a transfer of a proton from one part of the substrate of the enzyme to another part. The overall aim is to discover how this magnesium-utilizing enzyme works. The key to this discovery lies in locating the atomic positions of hydrogen via neutron diffraction. The general information obtained with this technique will be useful for many

magnesium-containing enzymes (such as those involved in signal transduction) that are important in cancer research. A significant milestone on the path to achieving these goals was attained with the commissioning of the PCS at LANSCE and subsequently with the collection of neutron-diffraction data from a D-xylose isomerase protein crystal by the Oak Ridge/Fox Chase team with help from scientists from the Bioscience Division. The data obtained on the PCS were beyond the team's greatest expectations for quality and nearly equals their best cryogenic ultra-high resolution x-ray data to date.

Despite some early problems that necessitated a nineteen-hour nonstop cross-country drive to provide more crystals for the PCS experiment, data collection has since been progressing steadily. The crystal shown in Fig. 1 is positioned in the neutron beam, and diffraction data are collected over a 36- to 48-hour period. The crystal is then moved 30°, and the process is repeated. In all, the Oak Ridge/Fox Chase team expects to collect data from twelve different crystal positions. Although their original plan was to collect neutron diffraction data to about 2 Å, the crystal used on the PCS has produced high-quality data to a 1-Å resolution, nearly equaling the resolution of their x-ray data collected at the APS using flash-cooled crystals. The team determined their best high-resolution x-ray reflections at 1 Å to have a signal-to-noise ratio of approximately 4 to 1. But with the neutron data collected on the PCS, they found a number of reflections at this same resolution to have a ratio of 30 to 1. The team plans to complete their data collection during the 2002 run cycle and to finish the structure analysis by next summer.

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